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Metastable Supramolecular Polymer Nanoparticles via Intramolecular Collapse of Single Polymer Chains

E. Johan Foster, Erik B. Berda, and E. W. Meijer*

Institute for Complex Molecular Systems and Laboratory of Macromolecular and Organic Chemistry, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven, The Netherlands

Received March 4, 2009; E-mail: e.w.meijer@tue.nl

The emergent field of nanotechnology requires the facile preparation of well-defined shape-persistent three-dimensional nanometer-sized objects. Polymeric nanoparticles (PNPs) continue to receive intensive research efforts for these ends.[1−13] The synthesis of PNPs through the intramolecular cross-linking and collapse of single polymer chains is currently being investigated as a simple and facile route to discrete nanometer-sized objects without the need for selective solvent techniques.[2,5,6,11] Drawing from the elegance of nature's own chemistry the application of noncovalent bonding to materials synthesis permits the fabrication of unique and highly defined architectures.[14] Invoking this concept in the collapse of single polymer chains by replacing covalent cross-links with supramolecular cross-links provides reversibility to this intramolecular collapse and ultimately control over the polymer conformation.[4]

Carefully selecting the species that will effect the supramolecular cross-linking is critical. Based on our previous work[14,15] we chose the 2-ureido-pyrimidinone (UPy) group linked to the polymer through urea or urethane moieties. Coupling the two combines the high dimerization constant of UPy with the added stability of the urea or urethane and their proven ability to form highly defined aggregates and fibers. While other reports in this area rely on selective solvent techniques to induce the collapse of a single chain by hydrogen bonding,[4] we sought to devise a method analogous to the covalent strategy where the cross-linking is carried out in a single step on a dilute solution of the precursor polymer.[2,5,6,11] By protecting the terminal carbonyl site on the UPy group with *o*-nitrobenzyl chloride we have effectively decreased the association constant such that photo-deprotection promotes UPy dimerization,[16] gel formation in concentrated solutions, and chain collapse into nanoparticles when sufficiently dilute.

The examples discussed here are substituted poly(norbornenes) bearing protected UPy urea (**1**) or UPy urethane (**2**) pendants. Polymer **1** is decorated with alkyl substituents simply to overcome solubility problems, which were not encountered in the synthesis of **2**. These polymers were synthesized at concentrations of ∼5 mg/mL of monomer in CH_2Cl_2 by ROMP using second generation Grubbs' catalyst. UPy-pendant group concentrations of 20 mol % (**a**) and 10 mol % (**b**) were examined, and molecular weights range from 163 kDa to 207 kDa with polydispersity indices from 1.49 to 1.81. Detailed molecular weight data for these protected polymers can be found in the Supporting Information (SI). We initially choose the work with norbornenes because ROMP is one of the most efficient methods available for the synthesis of functionalized polymers.[7,13,17−23] While these examples have very specific structures, this method is general and has been successfully applied to a variety of additional systems as well.[24]

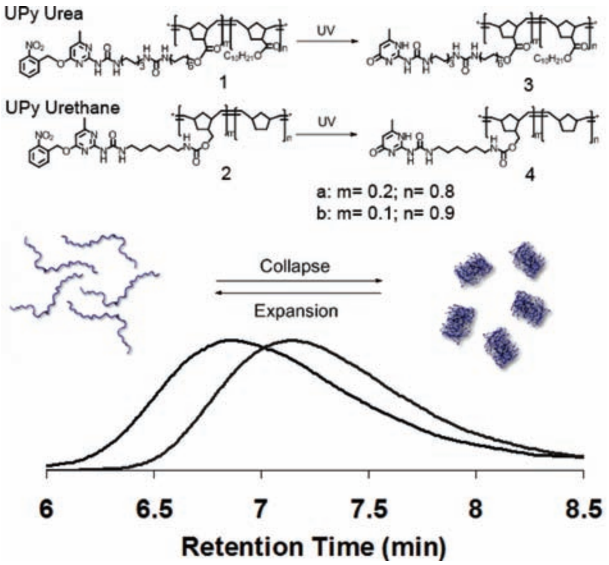

Figure 1. Nanoparticle fabrication for polymers **3a,b** and **4a,b**; the collapse by UV irradiation and expansion on the addition of acid. The GPC curves illustrate the collapsed and expanded state of polymer **3b**.

Table 1. Collapse and Expansion GPC Data for Polymer **1b** → **3b**

cross-linking time (h)	M_n[a] (g/mol)	M_w/M_n	% change
0	207 000	1.55	-
1	175 000	1.53	15
2	153 000	1.54	26
2 followed by acidification	193 000	1.64	7

[a] Determined by GPC in $CHCl_3$ versus polystyrene standards.

Figure 1 illustrates the method for collapsing the single chains at concentrations below 1 mg/mL in $CHCl_3$. The deprotected polymers **3** and **4** show a decrease in hydrodynamic volume upon irradiation with 350 nm light as evident by the increased retention time in a GPC experiment.[25] Polymers with 10 mol % UPy incorporation show a 20% decrease in apparent molecular weight after deprotection with no noticeable difference between UPy urea or UPy urethane moieties. At higher cross-linker concentrations, the apparent decrease in molecular weights for the UPy urea and UPy urethane groups are 26% and 34%, respectively. Acidification of the sample (formic acid, 1−4 μL/mL) disrupts the hydrogen bonding resulting in the expansion from a nanoparticle into a coil, demonstrating the reversibility of this state. Table 1 depicts typical GPC data for this intramolecular collapse for polymer **3b**. The concentration upon which the intramolecular collapse is favored over intermolecular cross-linking is below the C*-concentration of the protected polymer **1b**, but much higher than expected for excluding the network formation, probably due to the reversible nature of the cross-linking. The latter is only observed at concentrations above 10 mg/mL yielding highly viscous solutions.

When a film of these collapsed nanoparticles (**3** or **4**) is cast from $CHCl_3$ solutions on glass, the film remains completely soluble

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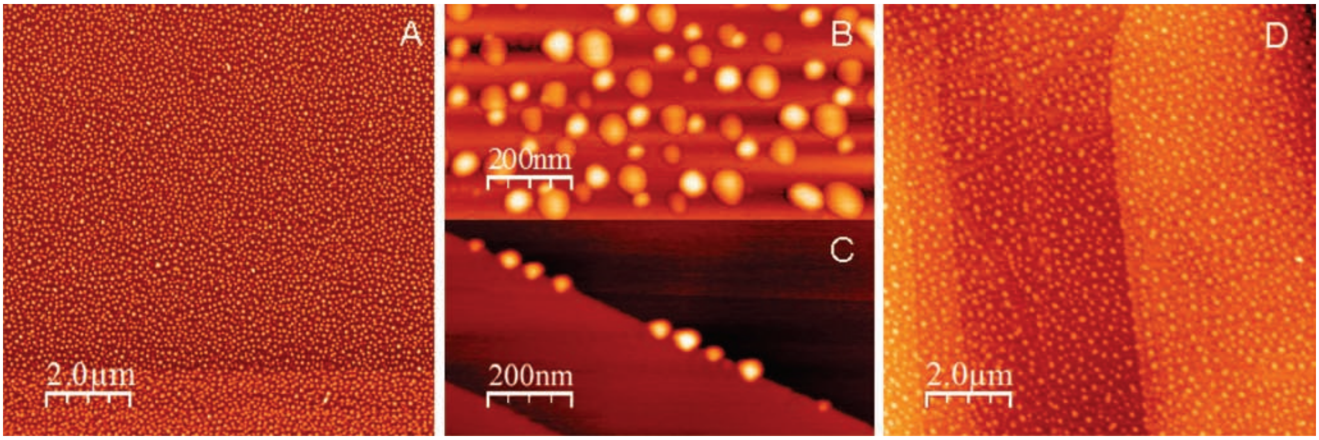

Figure 2. AFM images of single-chain nanoparticles. Panels A and B are polymer **3b** on mica, while panels C and D are polymer **4a** on graphite. See the text for more explanation of individual panels.

in $CHCl_3$. However, upon heating the neat film for 20 min at 80 °C, the film becomes essentially insoluble (see SI) as the result of the formation of an insoluble supramolecularly cross-linked network. These results demonstrate the metastability of the collapsed nanoparticle state. Ultimately this work presents a new route to an elusive target: a solution processable metastable formulation that can be turned into a high-performance supramolecular material.

Visualization of the particles on mica and graphite surfaces by AFM is shown in Figure 2. The concentration of sample preparation is critical to circumvent nanoparticle aggregation (see below). In panels A and D (**3a** on mica and **4a** on graphite, respectively; cast from $CHCl_3$ solutions: 2 μL, 0.002 mg/mL) a 10 × 10 μm^2 scanning area shows thousands of well-defined sphere-like particles with a narrow polydispersity. A blown up section of panel A (Figure 2B) more clearly displays the size of the particles of polymer **3a**: 2−3 nm in height with diameters of roughly 50 nm (without tip correction). Panel C shows the particles of polymer **4a** on graphite cast from a very dilute (2×10^{-4} mg/mL) solution; individual particles aligned on a groove of the surface can be seen. The pictures in Figure 2 are exemplary for all polymer nanoparticles studied, provided they are made under the right conditions.

By taking the shape of the particles to be half an ellipsoid, calculating the volume, and solving for the radius of a sphere, we can estimate the diameter of the corresponding spherical particles (see SI). The particles as shown in Figure 2 would have an estimated diameter of ~20 nm when in solution, which agrees well with the radius of gyration of polystyrene with molecular weight analogous to these collapsed poly(norbornenes) as deduced from GPC. This indicates that the particles are likely collapsed single chains in nature. Although the spheres seen in Figure 2 appear to be of somewhat different sizes, analysis of the size distribution of the particles measured by AFM (PDI of 1.44 using a Gaussian distribution of particle area in panel A) is consistent with the molecular weight distribution measured by GPC ($M_w/M_n = 1.54$). Hence this effect is attributed to the polydispersity of the polymer sample (see SI). While our particles are larger than those made through the covalent cross-linking approach (typically *ca.* 10 nm in diameter),[2,5,6,11] it is important to understand that our UPy cross-linked particles are less dense, owing to the size and steric requirements of the UPy urea or UPy urethane dimer and their possible stacking. Investigations to prove the existence of ordered stacks of UPy-urethane or UPy-urea dimers are ongoing, but the observed metastability is in agreement with a cooperative intramolecular supramolecular collapse. Whether we can compare these nanoparticles with side-chain induced folded macromolecules remains to be seen.

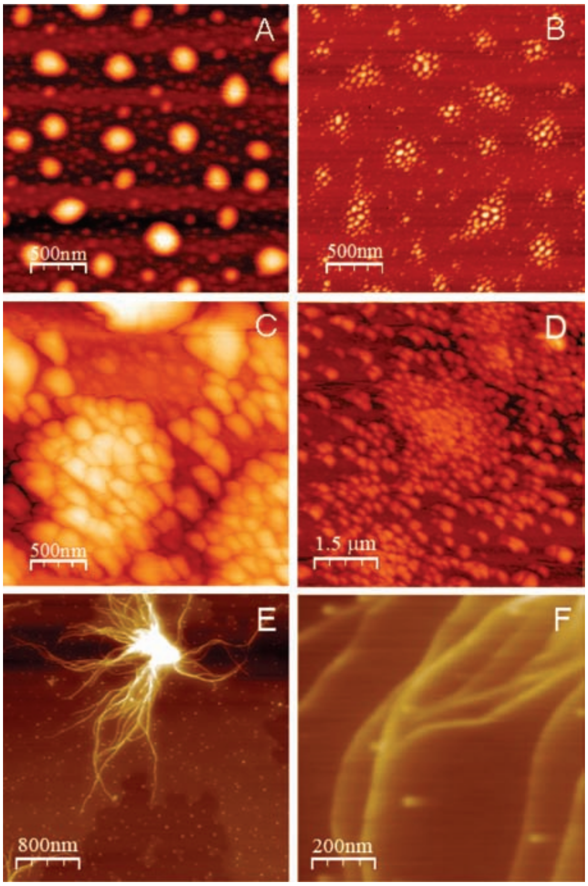

Figure 3. Aggregation of single-chain nanoparticles of polymer **4a** or **4b** in panels A−D. In panels E and F, specific areas of polymer **3a** are given. All AFM studies are on mica.

Similar to other synthetic nanoparticles or even to proteins, aggregation of our particles seems logical, since the particle surface will present groups available for aggregation. To investigate this behavior we returned to AFM studies (Figure 3) and found intriguing results depending on polymer and sample preparation. Drop casting with slow evaporation of the solvent (2 μL, 0.002 mg/mL of of **3a** or **4a** in $CHCl_3$ solution, evaporated in a chamber saturated with $CHCl_3$ vapor) allows concentration gradients on the surface leading to formation of aggregates as the drop slowly concentrates. Scanning the surfaces reveals that single chains are deposited at the edges of the evaporating drops; as the drop concentrates, aggregates of 100−200 nm appear next to the single-chain nanoparticles (panel A), and it seems evident that they are formed from the latter (panel B). With further concentration these smaller aggregates conglomerate into huge cauliflower type arrays close to 1 μm in size (panel C) and even larger agglomerates (panel D). This type of aggregation behavior closely resembles previous reports on the fractal-like self-assembly of functionalized gold nanoparticles.[26]

When large quantities (10 μL) of the nanoparticles with the highest UPy-urea concentration (sample **3a**) are deposited on mica, aggregates with long tendril-like fibrils appear along with single particles. These fibers seem nucleated (panel E) and appear to be linear assemblies of the single-chain nanoparticles (panel F). We surmise that this is a direct result of the higher UPy-urea loading and the assumed fibrils present in the nanoparticle (a kind of beta-strand of the lateral stacking of UPy-urea dimers within a nanoparticle will then lead to a linear aggregate of these particles by extending the stacking beyond the particle). We are currently performing more detailed studies to fully understand these aggregation phenomena.

Preliminary DLS measurements agree well with the AFM results: single-chain nanoparticles 16 nm in diameter in extremely dilute regimes and aggregates on the order of 150 nm (**3a**) and 200 nm (**4a**) in diameter for more concentrated solutions. It should be noted that these larger particles scatter much more strongly than the single chains; it is therefore likely that single-chain particles are still present in the more concentrated samples. Upon sonication of the more concentrated solutions the larger aggregates disassemble again, indicating that the single-chain nanoparticles are not disintegrated during the aggregation process. This is in full agreement with the high solubility of cast films without thermal treatment (see above).

We have demonstrated a facile route to well-defined metastable polymer particles via the supramolecular intramolecular cross-linking of single polymer chains. The metastability and aggregation behavior is akin to nature's own chain collapsed nanoparticles, namely proteins and nucleic acids. By further applying our expertise in self-assembly we hope to impart further generations of these particles with controllable internal structures, not unlike beta strands or helices, and exploit this behavior to create nanostructures of increasing complexity in the interior as well as at the exterior, while simultaneously tailoring them for specific applications.

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Supporting Information Available: Detailed experimental procedures, synthesis and characterization data for polymers and nanoparticles, and additional AFM images. This material is available free of charge via the Internet at http://pubs.acs.org.

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